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The therapeutic and diagnostic role of exosomes in cardiovascular diseases[☆]

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ABSTRACT

Exosomes are nano-sized membranous vesicles that are secreted by cells. They have an important role in transferring proteins, mRNA, miRNA and other bioactive molecules between cells and regulate gene expression in recipient cells. Therefore, exosomes are a mechanism by which communication between cells is achieved and they are involved in a wide range of physiological processes, especially those requiring cell–cell communication. In the cardiovascular system, exosomes are associated with endothelial cells, cardiac myocytes, vascular cells, stem and progenitor cells, and play an essential role in development, injury and disease of the cardiovascular system. In recent years, accumulating evidence implicates exosomes in the development and progression of cardiovascular disease. Additionally, exosomal microRNAs are considered to be key players in cardiac regeneration and confer cardioprotective and regenerative properties on both cardiac and non-cardiac cells and, additionally, stem and progenitor cells. Notably, miRNAs may be isolated from blood and offer a potential source of novel diagnostic and prognostic biomarkers for cardiovascular disease. In this review, we summarize and assess the functional roles of exosomes in cardiovascular physiology, cell-to-cell communication and cardio-protective effects in cardiovascular disease.

Introduction

According to the World Health Organization (WHO), cardiovascular diseases (CVDs) represent the leading cause of death worldwide in both males and females (http://www.who.int/ mediacentre/factsheets/fs317/en/). The heart itself consists of many different types of cells, including cardiomyocytes, vascular cells, cardiac fibroblasts and neural cells. To maintain effective organ development and function, efficient interaction between these various cell types is essential [1]. Communication must coordinate the function of a wide variety of cell types; these include endothelial cells, smooth muscle cells, fibroblasts, immune system-related cells, pluripotent cardiac stem cells and cells derived from other connective tissue compartments [1–3]. Multidirectional cross talk between cells, both local and distant, can be mediated by various hypertrophic stimuli such as paracrine/autocrine signals, growth factors and other humoral mediators, direct cell–cell interactions, gap junction-mediated contacts between cells, signaling transduction through adhesion molecules including integrins and indirect interaction via extracellular matrix (ECM) [3–6]. In recent years, a group of small extracellular vesicles (exosomes), ranging in size from 40 to 100 nm and naturally released from mammalian cells, have been identified. Much evidence supports the role of these extracellular vesicles in cell–cell communication between different

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cell types, and they are therefore central to cardiac cell–cell communication [7–9]. Exosomes are secreted into the extracellular space and contain a variety of bioactive molecules such as cytoplasmic proteins, membrane proteins, cytokines, chemokines, cellular receptors, non-coding RNA, plus mRNA and miRNAs [10,11]. Extensive research shows that exosomes contribute to both normal physiology (for example, cardiac development, reticulocyte maturation and myocardial angiogenesis), as well as to pathophysiological processes including development of cardiovascular disease [12–14]. Several reports suggest that exosomes have potential in the clinical realm, with use in diagnosis, prognosis and therapeutics [15]. At present, hundreds of circulating forms of miRNA have been identified in the blood. This population of miRNAs can exist in both exosomal and non-exosomal forms. In recent years, the exosomal miRNAs have attracted much attention as novel disease biomarkers and useful diagnostic tools with high specificity compared to conventional biomarkers [15]. Here, we evaluate the functional roles of exosomes and exosomal miRNAs secreted by different cells in the cardiovascular system; in addition, the possibilities for use of exosomal miRNAs for cardioprotection and as biomarkers (in diagnosis, prognosis, and therapeutic benefit) in cardiovascular disease are also explored.

Extracellular vesicles (EVs)

The extracellular space of multicellular organisms contains various types of metabolites, including ions, proteins, lipids and polysaccharides. The extracellular space also contains a heterogeneous group of membranous vesicles termed "extracellular vesicles" (EVs) that originate from the endosome or plasma membrane, range in size from 30 to 1000 nm in size and carry a variety of cargos [16,17]. To date, several types of EVs have been described, including exosomes, shedding microvesicles (MVs) and apoptotic bodies (ABs). No definitive criteria have been set to classify the different types of vesicles; however, EVs can be categorized based on differences in biogenesis or size. EVs can be released through the outward budding or inward budding of the plasma membrane [18]. The outward budding of endosomal membrane results in the formation of shedding microvesicles (MVs) that are large membrane-limited vesicles (considered to be larger than 100 nm) that are shed from the plasma membrane of a wide variety of cell types [19]. In contrast, the inward budding of endosomal membranes results in the formation and accumulation of intraluminal vesicles (ILVs) within large multivesicular bodies (MVBs) that ultimately result in the release of exosomes, 40–100 nm diameter membranous vesicles, through fusion with the plasma membrane [18]. ABs are the largest EVs ranging in size from 1 to 5 µM and released by apoptotic cells via blebbing of the plasma membrane [20]. In this review, we will focus on the biogenesis of exosomes, and their cargo loading, content, release, and uptake as an important group of EVs.

Exosome biogenesis

Exosomes, defined as small membrane vesicles originating from intracellular compartments, have garnered much interest in recent years [18]. Exosomes were originally described by Johnstone et al. in 1983 when, during investigation of reticulocyte maturation, they described the secretion of small endosome-derived vesicles [21]. As noted above, unlike MVs that are formed by an outward budding of the cell membrane, exosomes are usually generated within the endosomal network of the cell. Exosomes are formed by inward budding of the cell membrane forming early endosomes; this is followed by a second inward budding of the endosomal membrane, leading to formation of late endosomes that contain the various ILVs and are known as MVBs [9]. Intracellular MVBs have different fates based on their biochemical properties. They can undergo degradation by direct fusion to lysosomes and consequent degradation of the MVBs (degradative MVBs). Another option is for MVBs to fuse with the cell membrane, thus releasing the ILVs into the extracellular environment (exocytic MVBs). ILVs released into the extracellular compartment are termed 'exosomes' (Fig. 1) [22].

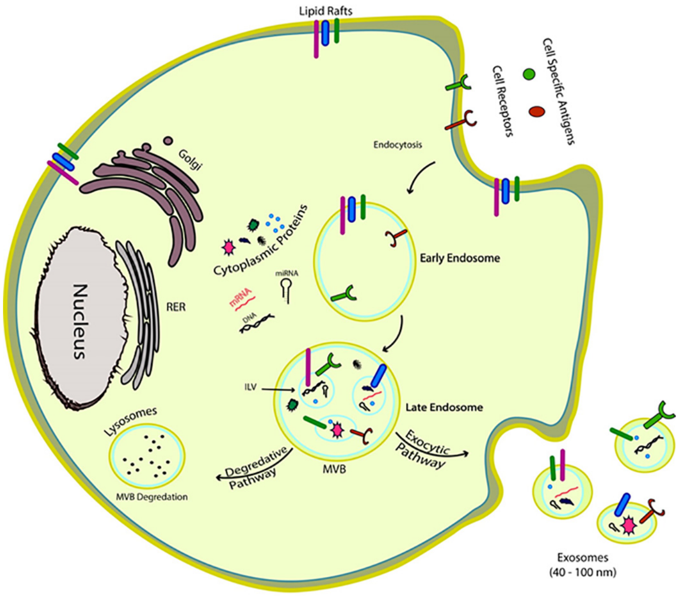

Fig. 1. The generation of exosomes. Biogenesis of exosomes begins with inward budding of the cell membrane, to form early endosomes. Specific membrane proteins and receptors can incorporate in the endosytotic endosomes. Subsequently, a second inward budding of the endosomal membrane occurs, leading to formation of late endosomes which are then transformed into multivesicle bodies (MVBs) that contain the various intraluminal vesicles (ILVs). MVBs can undergo degradation by direct fusion to the lysosomes. Alternatively, MVBs may fuse with the cell membrane and release ILVs into the extracellular space as exosomes.

Sorting proteins in exosomes

The exosomal membranes contain various components, such as lipid raft microdomains enriched with cholesterol, sphingomyelin, glycolipids and ceramide, and may include similar surface proteins to the cell from which they are derived [23]. To date, a number of different molecular mechanisms of exosome formation and specific sorting of proteins into these vesicles have been defined [24]. The Endosomal Sorting Complex Required for Transport (ESCRT) is a multiprotein complex that consists of four proteins, ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III, as well as other associated proteins having a key role in the biogenesis and degradation of MVBs in an ubiquitin-dependent manner [9,25]. ESCRT-0 participates in cargo sorting in an ubiquitin-dependent manner; ESCRT-I and ESCRT-II are involved in membrane budding; ESCRT-III is responsible for splitting of the vesicle from the membrane, and other associated proteins (particularly the VPS4 ATPase) mediate the separation and recycling of the ESCRT machinery [9]. Moreover, ESCRT- independent mechanisms are also known to play a role in exosome biogenesis [26,27]. The tetraspanins CD9, CD63 and CD81, through interaction with other transmembrane proteins, cytosolic proteins and lipids, play an important role in the composition of ESCRT-independent loading into exosomes [24]. Very specialized mechanisms, including ESCRT dependent and independent mechanisms for selective sorting of proteins into ILV and exosomes, result in the formation of heterogeneous populations of MVBs and exosomes [24].

Release of exosomes

Exosomes release into the extracellular space is facilitated by the fusion of membrane of the MVB with the plasma membrane. Exosomal release from the cell may be constitutive or inducible. The constitutive pathway is regulated by several types of proteins, RAB GTPases (Rab27a/b, Rab11 and Rab35), WNT5A, glycosphingolipids, hetrotrimetric G-protein and flotillins, for example [28–31]. The inducible release pathway can be triggered by stimuli such as DNA damage, hypoxia, aberrant intracellular calcium release, cytokines and other cellular stresses [32–36].

Composition of exosomes

Abundant evidence indicates that a wide range of compounds are transported within exosomes and, generally, these can be categorized into three major groups: proteins, lipids, and nucleic acids. These molecules can be common between exosomes or may comprise a specific set of compounds in a specific type of exosome [37]. The common exosomal cargoes that have been identified are cytoskeletal elements (actin, myosin, tubulin), tetraspanins (CD81, CD82 CD63, and CD9) and receptors such as Tumor Necrosis Factor Receptor 1 (TNFR1), enzyme and molecular chaperones (HSP90, HSP70, Hsp60, Hsp20, αB-Crystalline), proteins involved in exosome biogenesis (ESCRT complex, i.e. Tsg101, Alix) and signaling molecules that are found in cell membranes or cytosol of their cell of origin (examples being kinases, GTPase Hras, RhoA, RAP1B and Guanine nucleotide-binding protein subunit -G proteins) [37,38]. Considerable evidence now indicates that exosomes additionally contain specific compounds, for example double-stranded DNA, mRNA and noncoding RNA (microRNA and lncRNA) [39–41]. Recent studies indicate that exosomal mRNA and microRNA play a crucial role in efficient inter-cellular communication from inside a donor cell to a recipient cell. Proteins and microRNA may also be considered as potential biomarkers for cancer and other pathological conditions [42].

Functional role of exosomal miRNA in intercellular communication in the cardiovascular system

MicroRNAs (miRNAs) are noncoding single stranded RNA molecules that serve as key regulators in RNA silencing and regulation of expression of their target genes via mRNA degradation or repression of protein translation [43]. Studies show that miRNAs act as fundamental regulators of intercellular communication during human physiological and pathological processes [44–49] and are affected by several types of pharmacological agents [50–53]. Altered miRNA expression profiles are associated with cardiovascular disease, including atherosclerosis, myocardial infarction, coronary artery disease, heart failure and cardiac arrhythmias [54–56]. Interestingly, miRNAs can be actively transported by becoming entrapped in exosomes and, therefore, are protected from degradation by RNases. Numerous studies suggest that exosomal miRNAs may function as important signaling mediators outwith the cell in which they were produced [44,57,58].

In recent years, it has been observed that exosomes secreted by cardiac, endothelial and vascular cells harbor a variety of miRNAs which may be transferred to recipient cells and modulate their function. These exosomal miRNAs mediate intercellular communication in the heart and vascular system and also between endothelial cells and cardiac myocytes, endothelial cells and smooth muscle cells, fibroblasts and cardiac myocytes [44,59–61]. As mentioned in Table 1, exosomal miRNAs play an indispensable role in the regulation of cellular functions in cardiovascular systems.

Here, we explore the potential roles of exosomal miRNAs in the regulation of intercellular communication in some of cardiovascular diseases.

Exosomal miRNA function in myocardial vascular deficiency

miRNA-320, a well-known anti-angiogenic miRNA, targets angiogenesis-related genes, especially IGF-1, Hsp20 and Ets2, in endothelial cells [62]. Several studies indicate that miRNA-320 can regulate the function of endothelial cells as well as angiogenesis in diabetes [63,64]. Recently, it was reported that diabetic cardiomyocyte-derived exosome-encapsulated miR-320 exerts an anti-angiogenic effect in a model of type 2 diabetes in rats by inhibiting endothelial cell proliferation, migration and tube-like formation [61]. Wang et al. observed that exosomes derived from cardiomyocytes isolated from type 2 diabetic rats encapsulated higher levels of miRNA-320. These exosomes were effectively taken up by cardiac endothelial cells and delivered miRNA to them. MiRNA-320 functionally down-regulated its target genes IGF-1, Hsp20 and Ets2 in recipient cardiac endothelial cells, leading to suppression of endothelial cell proliferation, migration and tube-like formation. These findings provide a new molecular mechanism underlying the diabetes-caused impairment of myocardial angiogenesis, which may be caused by anti-angiogenic signals from cardiomyocytes via exosomes, but not from endothelial cells [61].

Exosomal miRNA function in atherosclerosis pathology

Smooth muscle cells are important in the development of myocardial infarction. Recently, miRNA-143 and miRNA145 were shown to modulate the fate of smooth muscle cells as well as their plasticity [65]. In a study carried out by Hergenreider et al., exosomes enriched with miRNA-143 and miRNA145 were shown to modulate gene expression in smooth muscle cells and induce an atheroprotective effect via reduction of atherosclerotic lesion formation in an atherosclerotic mouse model [44]. These results show that communication between endothelial cells and smooth muscle cells through an exosomal miRNA mechanism may be important strategy in the inhibition of atherosclerosis.

Exosomal miRNA function in heart failure

Recent reports have shown that exosomes derived from cardiac fibroblasts and containing a high concentration of miR-21* act as intercellular signaling molecules between cardiac fibroblasts and cardiomyocytes in cardiac hypertrophy. Consistent with these results, miR21*, a passenger strand microRNA which is usually degraded inside the cytoplasm during miRNA biogenesis, was exported by exosomes from the cardiac fibroblasts to neighboring cells. These data suggest that exosomes enriched with miRnA-21* are transferred to cardiomyocytes and affect target genes such as sorbin and SH3 domain-containing protein 2 (SORBS2) and PDZ and LIM domain 5 (PDLIM5), thus leading to cellular hypertrophy. These data show that inhibition of miR-21* by “antagomiR-21*” attenuated the development of cardiac hypertrophy in this mouse model. These findings on the role of miRNA-enriched exosomes in cell to cell communication may offer new insights with regards to the mechanisms involved in the pathogenesis of cardiomyocyte hypertrophy [59].

Recent evidence has suggested that the transfer of miR-200a in adipocyte-derived exosomes into cardiomyocytes induced

Table 1
Exosomal miRNA and cardioprotective effects.

miRNA	Cell source	Target molecule	Type of CVD	Function	Reference
miRNA-320	Cardiomyocyte	Down-regulation of IGF-1, Hsp20 and Ets2	Diabetes mellitus-induced myocardial vascular deficiency	Inhibition of cardiac endothelial cells migration and tube formation, anti-angiogenic effect	[61]
miRNA-143/ miRNA145	Endothelial cells	Regulation of atheroprotective gene expression	Atherosclerosis	Reduced atherosclerotic lesion formation	[44]
miR-21*	Cardiac fibroblasts	SORBS2, PDLIM5	Cardiomyocyte hypertrophy	Induction of cardiomyocyte hypertrophy	[59]
miRNA-1a	Plasma of MI mice	Down-regulation of CXCR4	Myocardial infarction	BM-cell mobilization	[67]
miR- 200a	Adipose tissue	Decreased TSC1	Cardiomyocyte hypertrophy	Activation of mTOR signaling; Induction of cardiomyocyte hypertrophy	[66]
let-7b-5p	Pericardial fluid	Inhibition of TGFBR1	Ischemic heart disease	Promotion of angiogenesis	[68]
miR-22	MSCs	Methyl CpG binding protein 2 (Mecp2)	Ischemic heart disease	Anti-apoptotic effect; reduction of cardiac fibrosis	[79]
miRNA-19a	MSCs	PTEN and BIM	Ischemic heart disease	Activation of Akt and ERK signaling pathways	[80]
miRNA-21	MSCs	PTEN	Myocardial infarction	Activation of the Akt signaling pathway	[81]
MiRNA-451	CSCs	–	Acute ischemia/reperfusion	Reduction in apoptosis; protection from oxidative stress	[74]
miR-210, miR-132, and miR-146a-3p	CSCs	Down-regulation of ephrin A3 and PTP1b, RasGAP-p120	Myocardial infarction Ischemic heart disease	Anti-apoptotic and proangiogenic activities	[60]
miR-15b, miR-17, miR-20a, miR-103, miR-199a, miR-210, and miR-29	CSCs	Down-regulation of pro-fibrotic gene expression	Myocardial infarction	Pro-angiogenic effect	[83]
miR-21	CPCs	Inhibition of PDCD4	Ischemic heart disease	Anti-apoptotic effect; Protection of myocardial cells against oxidative stress	[85]
miR-146a	CDCs	Down-regulation of Irak-1 and Traf6	Myocardial infarction	Increased cardiomyocyte viability; protection against oxidant stress	[87]
miR-291, miR-294 and miR-295	ESCs	Enhanced expression of pluripotent markers OCT-4, SOX-2 and Nanog	Myocardial infarction	Increased neovascularization, cardiomyocyte proliferation and reduced fibrosis	[89]
miR-21 and miR-210	iPS	Inhibition of caspase 3/7 activation	Ischemic heart disease	Anti-apoptotic effect	[90]

phosphatase and tensin homolog (PTEN), cardiac stem cells (CSCs), cardiac progenitor cells (CPCs), mesenchymal stem cells (MSCs), cardiac progenitor cells (CPCs), programmed cell death 4 (PDCD4), cardiosphere-derived cells (CDCs), embryonic stem cells (ESCs), induced pluripotent stem cells (iPS).

Table 2
Exosomal miRNAs and proteins as biomarkers for CVDs.

Type of studyiCargo	Type of biomarker	Disease model	Type of study	Sample	Reference
miRNA	miR-1 miR-133a	Unstable angina pectoris, AMI	Animal model/ Human cell line	Serum/Human	[107]
	miR-1	Acute MI	Animal model/ Human	Urine/Rat	[108]
	miR-1 miR-21 miR-499	ACS	Human	Blood/Human	[111]
	miR-133b miR-208b miR-499	I/R injury, MI Langendorff	Animal model	Plasma/Mice Plasma/Pig	[112]
	miR-1, miR-24, miR-133a/b, miR-208a/b, miR-210	CABG	Human	Plasma/Human	[129]
	miR-192 miR-194 miR-34a	HF	Human	Serum/ Human	[130]
	miR–423–5p miR320a miR-22 miR-92b	HF	Human	Serum/ human	[119]
	miR-146a	PPCM	Animal model/ Human cell line/ Human	Plasma/ CKO mice	[45]
	miR- 223	Acute Ischemic Stroke	Human	Blood/ Human	[113]
Proteins	Collagen, fibronectin, lysyl oxidase like 2 (LOXL2)	Stress-induced endothelium cells	Human cell line	Human microvascular endothelial cells	[120]
	TNF-α	Hypoxia-induced cardiomyocyte	Animal model/ rat cell lines	–	[121]

cardiac hypertrophy. These findings demonstrate that a significant crosstalk between adipocytes and cardiomyocytes exists and is guided by the exosomal miR-200a, and that miR-200a was able to target genes involved in mTOR pathway regulation. These data show that activation of PPARγ in adipose tissue leads to expression and release of exosomal miR-200a into the vascular system. Consequently, exosomes containing miR-200a, via downregulation of TSC1 expression in recipient cardiomyocytes, promoted activation of mTOR signaling and resulted in cardiomyocyte hypertrophy [66].

Exosomal miRNA function in ischemic heart disease

A recent report showed that exosomal miRNA-1 mediates functional crosstalk between the ischemic heart and bone marrow (BM) cells. In mice with an acute myocardial infarction (MI), exosomal miRNA-1a was released into the blood stream and led to inhibition of CXCR4 expression in BM mononuclear cells. Based on previous studies, it is known that MI leads to mobilization of various BM cells, hence it seems that exosomal miR-1 may contribute to that BM-cell mobilization during ischemic injury [67].

Recent investigation indicates that pericardial fluid (PF) contains exosomes with a high concentration of cardiovascular-derived miRNAs, suggesting that exosomes are involved in the transportation of miRNAs between the heart and the PF. PF exosomes have been shown to be functionally active, and potentially improved survival, with increased proliferation and formation of capillary-like cellular networks and inhibited apoptosis in cultured endothelial cells. PF-derived exosomes demonstrate different behaviors dependent upon the differences in their miRNA content. Interestingly, let-7b-5p is highly expressed in the exosomes in the PF. These findings indicate that the proangiogenic miRNA, let-7b-5p, is transferred from the PF exosomes to the EC and leads to improvement of capillary-like tube formation and an increase in EC proliferation, resulting in enhanced angiogenesis *in vitro*. Further, increased let-7b-5p reduced the mRNA expression of TGFBR, an antiangiogenic factor, as a target gene of let-7b-5p in the EC [68].

Recently, the important role of serum exosomal proteins in regulating vascular and endothelial cell function in diabetes has been reported. Serum exosomes isolated from diabetic mice were taken up by aortic endothelial cells and led to profound endothelial dysfunction in nondiabetic mice. Data reveal that serum exosomes are enriched in arginase 1 protein (Arg1). Arg1 is the enzyme that regulates the level of L-arginine, the substrate for endothelial NOS-mediated NO production in endothelial cells. Serum exosomes entered endothelial cells and markedly impaired endothelial function through transfer of exosomal Arg1 causing reduced levels of NO in endothelial cells in obese and diabetic mice. This study demonstrated that serum exosomes containing Arg1 play an important role in cell-to-cell communication and regulation of cellular mechanisms during development of diabetic endothelial dysfunction. [69].

The role of exosomes secreted by stem cells in cardiovascular system

Over the last decade, extensive research has shown that stem cells have beneficial effects in cardiac regeneration through the release of factors that improve cardiac function by promoting differentiation of new cardiomyocytes. Since exosomes are considered to be the main transporters of paracrine factors, it appears that the exosomes secreted by different type of stem cells can contribute to this process and exert their cellular effects through regulation of the expression of specific genes [70]. For instance, exosomes secreted by CD34$^+$ stem cells have a therapeutic effect on angiogenesis, both *in vitro* and *in vivo* [71]. In a study conducted by Lai et al., MSCs derived from human embryonic stem cells were shown to provide cardioprotection during myocardial ischemia/reperfusion injury through the paracrine secretion of exosomes [72]. Cardiac progenitor cells (CPC) derived from adult myocardium have been touted as a very promising stem cell for delivering cardioprotection and repair due to several important properties of CPCs, such as self-renewal, clonogenicity, and multipotency [73]. Recent findings indicate that exosomes isolated from CPCs (CPC-exosomes) can be used therapeutically for cardioprotection in cardiac disease, such as acute ischemia/reperfusion (MI/R) injury, that leads to cardiomyocyte apoptosis and death [74]. This protective effect was evaluated *in vitro* and in mouse models of acute MI/R where delivery of CPC-exosomes was able to efficiently suppress cardiomyocyte apoptosis induced by oxidative stress both *in vitro* and *in vivo* [74].

Protective effect of stem cell-derived exosomal miRNAs in CVD

Stem cells can secrete miRNA-containing exosomes. miRNAs play an important role in stem cells via regulation of cell proliferation, neuronal differentiation and apoptosis [75,76]. Recent data indicates that exosomes play a major role in miRNA intercellular communication [77].

As mentioned in Table 1, stem cell-derived exosomal miRNAs have therapeutic potential in the treatment of cardiovascular diseases. In the following sections, we will summarize recent findings on the potential protective effects of stem cell-derived exosomal miRNAs in treatment of cardiovascular diseases.

Cardioprotective effects of exosomal miRNA derived from mesenchymal stem cells (MSCs)

Mesenchymal stem cells (MSCs) have potential therapeutic effects in the treatment of ischemic heart disease and their therapeutic potential may relate to exosomes [78]. In a study reported by Feng et al., the exosomes released by MSCs from mouse bone marrow subjected to ischemic preconditioning were enriched with miR-22. The exosomal miRNA-22 exerts its anti-apoptotic effect by direct targeting of methyl CpG binding protein 2 (Mecp2) in cardiomyocytes that reported to be upregulated in the ischemic heart. These data show that the miR-22 induced reduction in apoptosis and amelioration of fibrosis, both being significant in the treatment of cardiac diseases. These findings indicate that exosomes released from MSCs could serve as important carriers of miRNA-22 for treatment of myocardial infarction [79].

In a recent study, the cardioprotective effect of exosomes derived from MSCs overexpressing GATA-4 was investigated in neonatal rat cardiomyocytes. The data revealed that the anti-apoptotic miRNA-19a was highly expressed in exosomes derived from MSCs overexpressing GATA-4. These exosomes transferred the miRNA-19a into cardiomyocytes via rapid internalization and could significantly improve ischemic myocardial function and promote myocardial protection. MiRNA-19a downregulated the expression of target proteins, such as PTEN and BIM, in cardiomyocytes and enhanced cell survival by activation of the Akt and ERK signaling pathways [80]. Therefore, MSC-derived exosomes may act as vehicles for the therapeutic delivery of miR-19a to ischemic myocardium for treatment of ischemic myocardium.

Recently, Wang et al. assessed the paracrine and therapeutic properties of endometrium-derived MSCs in a rat model of MI. Analysis of exosomal microRNAs indicated that the expression of miRNA-21 was selectively enhanced in exosomes from endometrium derived MSCs. *In vivo* assay demonstrated that endometrium-derived MSCs have a higher cardioprotective capability compared with bone marrow or adipose-derived MSCs. This property seems to relate to the presence of exosomal miRNA-21 in endometrium derived MSCs. To validate the cardioprotective property of miRNA-21 in adipose-derived MSCs, cardiomyocytes were treated with a miR-21 antagonist. The antiapoptotic and angiogenic effects of exosomes purified from endometrium-derived MSCs were abrogated in the cardiomyocytes transfected with the miR-21 inhibitor; effects were shown on PTEN, as a miR-21 target protein and downstream element of the Akt pathway. These finding suggest the potential of miRNA-21 for cardiovascular cell therapy and, also, that the endometrium may be considered as a source of MSCs for treatment of cardiovascular diseases [81].

Cardioprotective effects of exosomal miRNAs derived from cardiac stem cells (CSCs)

As noted earlier, cardiac stem cells (CSCs) can protect cardiomyocytes from oxidative stress via paracrine effects induced by exosomes. Chen et al. reported that CPCs have a high level of GATA4 expression, an essential transcription factor for heart development [82]. MiRNA-451 is one of the GATA4-responsive miRNAs in the miR-144/451 cluster, and this was significantly enriched in exosomes secreted by CPCs. Administration of purified CPC-exosomes protected cardiomyocytes from oxidative stress. This finding suggests that CPC-exosomes hold potential for cardioprotection in patients with a high risk of ischemia [74]. A comparison of the miRNA profiles of extracellular vesicles secreted by CPCs indicates that several miRNAs, including miR-210, miR-132, and miR-146a-3p, are markedly enriched in these extracellular vesicles (most of which were determined to be exosomes). Data demonstrate that miR-210 and miR-132 have anti-apoptotic and pro-angiogenic activities in the HL-1 cardiomyocytic cell line. Downregulation of ephrin A3 and PTP1b, as miR-210 validated targets, leads to inhibition of apoptosis in cardiomyocytic cells. MiR-132 stimulated and enhanced tube formation in HUVECs by targeting and downregulating RasGAP-p120 in the endothelial cells. Hence, extracellular vesicles secreted by human CPCs, via transport of miR-210 and miR-132, induce anti-apoptotic and pro-angiogenic effects and can thus improve cardiac function following myocardial infarction [60]. Exosomes secreted by CPCs grown under hypoxic conditions showed upregulation of miR-15b, miR-17, miR-20a, miR-103, miR-199a, miR-210, and miR-29 compared to exosomes secreted by CPCs under normal conditions. This indicates that hypoxic exosomes have pro-angiogenic effects and induced tube formation. The angiogenic effects of hypoxic exosomes was reduced by RNA-induced silencing complex (RISC) suppression, indicating that the presence of miRNAs in hypoxic exosomes are essential for modulating physiological effects. In the defective heart, fibroblasts are stimulated by cytokines such as TGF-β, which leads to enhancement of fibrosis. In addition, the hypoxic CPC-derived exosomes decreased pro-fibrotic gene expression, due to elevated levels of exosomal miRNAs that dynamically regulate genes involved in the fibrosis pathway [83]. In the ischemic zone of ischemic cardiovascular diseases, such as acute myocardial infarction, heart failure and myocardial ischemia/reperfusion injury, there is an increase in production of reactive oxygen species (ROS) and a reduction of antioxidant reserve; consequently, elevated oxidative stress in cardiac and vascular myocytes results in cardiomyocyte apoptosis and death [84]. Dhalla et al. reported that the amount of miR-21 was significantly upregulated in CPC-derived exosomes under oxidative stress and that these exosomes were able to protect myocardial cells against oxidative stress in an ischemic myocardial injury model. MiRNA-21 has been reported to contribute to gene regulation and cellular injury under oxidative stress. These findings show that exosomal miRNA-21, by targeting and downregulating programmed cell death 4 (PDCD4), has a crucial role in the apoptosis pathway in H9C2 cells [85].

Cardioprotective effects of exosomal miRNAs derived from cardiosphere-derived cells (CDCs)

Cardiosphere-derived cells (CDCs) have been shown to stimulate therapeutic regeneration and angiogenesis and to functionally improve infarcted myocardium, and exosomes may be involved in this process. Recently, the safety and efficacy of CDC derived exosomes were evaluated for development of a viable therapy based on exosomes for the repair of damaged cardiac tissue in heart failure patients [86]. MiR-146a was enriched in exosomes secreted by CDCs and conferred protective effects in a murine model of myocardial infarction. Data show that the miR-146a contained in CDC derived exosomes downregulated Irak-1 and Traf6, two proteins that are involved in the toll-like receptor signaling pathway, and play a major role in reduction of proinflammatory cytokines [87].

Cardioprotective effects of exosomal miRNAs derived from pluripotent stem cells

Pluripotent stem cells (both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) show promise for cardiac regeneration because of their inherent plasticity and high differentiation ability [88]. Khan et al. investigated ESC-derived exosomes for the repair of ischemic myocardium. ESC-derived exosomes are significantly enriched with the miR-290 family, including miR-291, miR-294 and miR-295. Interestingly, ESC-derived exosomes stimulated and enhanced CPC survival, cell cycle progression and proliferation, increased neovascularization, cardiomyocyte proliferation and survival, and reduced fibrosis post infarction, likely due to the proliferative response of the cardiomyocytes. [89]. A recent study suggested that exosomes secreted by induced pluripotent stem cells (iPS) can transfer cytoprotective signals to cardiomyocytes. MiR-21 and miR-210 are considered to be two important cardioprotective miRNAs, which are regulated by the Nanog/Stat-3 signaling pathway and hypoxia-inducible factor-1 α (HIF-1α), respectively. The levels of miR-21 and miR-210 were significantly increased in iPS-exosomes. The transfer of iPS-exosomes to H9C2 cells led to inhibition of caspase 3/7 activation and protected the cells from H_2O_2-induced oxidative stress. The *in vivo* anti-apoptotic effects of iPS-exosomes were evaluated in a mouse model of acute myocardial ischemia/reperfusion (MIR). The findings suggested that iPS-exosomes suppressed apoptosis by reducing activated caspase 3 proteins in the ischemic myocardium, thereby leading to enhanced cytoprotective effects [90].

Collectively, the above evidence indicates that exosomes from several different cell sources can be used as a therapeutic vehicles for treatment of cardiac diseases and highlights a new perspective on novel treatment methods in cardiovascular disease. However, use of exosomes as a novel therapeutic approach merits further investigation and understanding into how best to optimize their application for the treatment of cardiovascular diseases with the use of stem cells in the future.

The role of exosomal long non-coding RNAs (lncRNAs) in CVD

LncRNAs are a heterogeneous group of non-coding RNAs which are up to 200 nucleotides long and play significant regulatory roles in gene expression. Extensive research has shown that lncRNAs are involved in cardiovascular development and disease [91]. LncRNAs are able to regulate the functions of vascular and endothelial cells; hence, the dysregulation of certain lncRNAs has been associated with development of cardiovascular disease, including progression of atherosclerosis, coronary artery disease (CAD), MI, ischemia, and heart failure [92]. Recently, it was reported that exosomes are highly enriched in lncRNAs [93]. Chen et al. reported the importance of exosomal lncRNA GAS5 in atherogenesis. Their findings reveal that the exosomal lncRNA GAS5 is significantly increased in the atherosclerotic plaque collected from patients and animal models and inhibited the apoptosis of endothelial cells. They suggested that inhibiting the lncRNA GAS5 might be an effective therapy for atherosclerosis [94]. Wang et al. indicated that exosomal lncRNA HIF1A-AS1 plays a crucial role in atherosclerosis, the level of exosomal lncRNA HIF1A-AS1 being remarkably higher in patients with atherosclerosis compared with healthy subjects. Hence, exosomal lncRNA HIF1A-AS1 may serve as a potential biomarker for atherosclerosis [14]. Recently, using microarray analysis, lncRNA AC100865.1 (referred to as CoroMarker) was identified in the EVs, and could serve as a novel biomarker for predicting coronary artery disease (CAD) [95].

Therapeutic potential of cardiac exosomes

Considerable evidence acquired from research on exosomes indicates that they may be used as a therapeutic drug and nucleic acid delivery systems [96–98]. Exosomes are used physiologically as delivery vehicles for intercellular transport and delivery of nucleic acids and proteins. Moreover, some of their biophysical properties, such as straightforward isolation and characterization and manipulation of their content (RNA and protein), makes them attractive pharmaceutical delivery agents [58]. Exosomes are “natural liposomes;” hence, the use of exosomes to target specific cells, and to deliver various materials is essentially similar to the liposome-based technique already used in drug delivery systems [99,100]. Exosome-mediated transfer of specific proteins has been demonstrated by engineering CD34+stem cells that were able to secrete exosomes containing the pro-angiogenic factor, sonic hedgehog (Shh). Injection of modified CD34Shh cells have been used for treatment of mice with acute myocardial infarction leading to protection against ventricular dilation and cardiac functional declines as well as reduced infarct size, increased capillary density and improved long-term functional recovery [101]. The therapeutic potential of exosome-mediated siRNA delivery was demonstrated in Alzheimer’s disease in the brains of mice. Targeting was achieved by engineering exosome surface proteins loaded with exogenous siRNA to specifically target neuron cells. These exosomes were able to deliver siRNA across the blood–brain barrier and specifically target neurons, microglia, and oligodendrocytes [102]. A similar strategy can be employed to engineer exosome surface proteins to specifically target cardiac cells and reduce unwanted off-target effects of therapies. Exosomes can also serve as a drug carrier and thereby improve drug efficacy. Sun et al. used exosomes to deliver curcumin, an anti-inflammatory agent. Data indicate that use of exosomes as a delivery system can improve bioavailability, solubility, and stability of curcumin in the blood. Also, exosomal curcumin reduced the levels of inflammatory cytokines IL-6 and TNF-α in a mouse model [103]. Therefore, the use of exosomes as a drug delivery vehicle to overcome poor drug bioavailability creates new therapeutic opportunities for treatment of many different diseases without unwanted side effects.

Exosomes as diagnostic markers in cardiovascular disease

Another important feature of exosomes for clinical use involves their potential utility as biomarkers [104,105]. Exosomes contain a wide variety of compounds, such as proteins, lipids, mRNAs and miRNAs, that are representative of their cellular origin and reflective of the pathological condition of the origin cell. The cargo content of exosomes plays a major role in various pathological conditions and, therefore, these compounds may serve as non-invasive diagnostic biomarkers in biological fluids. The content and number of exosomes in the cardiovascular system varies under different pathophysiological conditions. For example, miRNAs and proteins in exosomes vary widely, and since cardiac-specific miRNAs are crucially participates in the regulation of cardiogenesis and heart function, hence exosomes may therefore serve as novel biomarkers.

Exosomal miRNAs

Extracellular circulating miRNAs are found in body fluids like blood. In addition, exosomes and other extracellular vesicles are the main source of circulating miRNAs. Therefore, exosomal miRNAs have great potential as diagnostic biomarkers for cardiovascular diseases.

Since injured cardiomyocytes release exosomes containing cardiac-specific miRNAs, such as miRNA-1 and miRNA-133a, changes in cardiac miRANs are found in the circulation of patients with Acute Coronary Syndromes (ACS) and Myocardial Infarction (MI) [106,107]. Studies show that the levels of miRNA-1 and miRNA-133a in serum samples of patients with unstable angina pectoris were increased following release from infarcted and peri-infarcted myocardium. These results indicate that levels of circulating miR-133a in patients with CVD mainly increased following myocardium injury and may serve as a biomarker for cardiomyocyte death [107]. High levels of miR-1 were also found in serum and urine exosomes in acute myocardial infarction (AMI) patients. Reported data demonstrated that urine miR-1 was significantly upregulated in rats following AMI and thus provided a urine biomarker of ischemic heart disease [108]. The diagnosis of AMI by circulating cardiac-specific miRNAs has a higher sensitivity and specificity than troponin T [109]. In addition, miRNAs are much more quickly detected in the circulation than cardiac ischemic biomarkers (troponin I and troponin T) [110]. MiRNA-1 and miRNA-133a exhibit the highest plasma levels for 2.5 h after the onset of MI symptoms [107,110].

Several other miRNAs including miRNA-1, miRNA-21, miRNA-146a, miRNA-208, miRNA-499 were found in a large cohort to be predictive diagnostic biomarkers for acute coronary syndrome [111]. Recently, Deddens et al. showed some isolated plasma-derived miRNAs, including miRNA-133b, miRNA-208b, and miRNA-499, were transported by extracellular vesicles in various models of I/R injury and are therefore potential biomarkers for MI [112]. MiRNAs can also be used to detect acute ischemic stroke, and exosomal miRNA-223 collected from blood samples of patients with ischemic stroke could represent as a novel biomarker [113].

Studies have also shown the specificity of some exosomal miRNAs in heart failure (HF) and suggested them as diagnostic biomarkers [114–116]. Matsumoto et al. showed that miRNA-34a, miRNA-192, and miRNA-194 are significantly increased in HF patients that developed following AMI, and confirmed in a cohort of 21 patients that these exosomal miRNAs could be considered as predictors of ischemic HF following AMI [116]. Halkein et al. showed that peripartum cardiomyopathy (PPCM) associated HF patients exhibited increased levels of circulating miRNA-146a. They also showed that the level of exosmic miR-146a was reduced in PPCM patients after standard treatment for HF [45]. MiRNA-92 is also closely linked to cardiovascular disease in many studies [117,118]. Goren at al. examined miRNA-92 correlation with HF patients and showed that the miRNA-92 level is higher in exosomes of HF patients [119].

Exosomal proteins

In addition to non-coding miRNAs, exosomes contain active proteins such as chemokines, heat shock proteins, immunoglobulin, growth factors and coagulation factors that are involved in signal transduction. Many studies have reported changes in these proteins in extracellular vesicles under hypoxic conditions, and they play an important role in the pathology of cardiovascular disease. Dejong et al. showed that exosomes secreted from cardiac cells under hypoxic conditions contain collagen, fibronectin, and lysyl oxidase like 2 (LOXL2) [120]. Yu et al. showed that exosomes secreted from hypoxia-rich cardiomyocytes contained upregulated TNF-a [121]. In addition, Pironti et al. showed that exosomes secreted from cardiomyocytes under high pressure contained high levels of angiotensin II (AngII) type 1 receptor (AT1R) that plays an important role in maintaining blood pressure and heart function [122]. By comparing three proteins isolated from the extracellular vesicles, polygenic immunoglobulin receptor (pIgR), complement factor C5a (C5a) and cystatin C, de Hoog et al. suggested that these three proteins are correlated with ACS disease [123]. Cheow et al. also suggested an upregulation in six proteins of extracellular vesicles in MI patients compared with stable angina patients. They reported upregulation of Complement C1q subcomponent subunit A (C1QA) and Complement C5 (C5), which probably incorporate into the complement activation pathway; Apolipoprotein D (APOD) and Apolipoprotein C-III (APOCC3), which are involved in lipoprotein metabolism; and Platelet glycoprotein Ib alpha chain (GP1BA) and platelet basic protein (PPBP) which act as alternatives to platelet activity [124].

The exosomal proteome can also provide a noninvasive method for diagnosis of cardiac allograft rejection. Kennel et al. showed that with heart transplant allograft rejection, changes occurred in the exosomal proteome, including adaptive immunity components, complement activation and coagulation [125]. Therefore, exosomal proteome analysis could be used as a novel biomarker to track acute transplant and allograft rejection.

Recently, Xu et al. identified microparticles (MPs), about 200 nm in size, that contain cardiac bridging integrator 1 (cBIN1) that could be released from transverse-tubules (t-tubules) of ventricular cardiomyocytes. cBIN1 creates membrane microfolds within the t-tubule membrane and helps to traffic ion channels and regulate local calmodulin concentrations. cBIN1 is present in human plasma, and its level is decreased in patients with heart failure. These findings indicate that the ESCRT-III pathway facilitates microparticle release from cardiomyocytes; therefore, the release of cBIN1 into the circulation may serve as a cardiac biomarker of heart failure [126]. This study showed that microparticles, in addition to exosomes, could be considered as important diagnostic biomarkers in CVD.

Although exosomal miRNA and other exosomal cargo may be considered as potential biomarkers, to date, the possible roles of exosomes in cardiac diseases have not yet been completely elucidated in terms of routine clinical practice. Therefore, further studies should be conducted to determine the diagnostic, prognostic and potential functional roles of exosomal content in cardiovascular diseases.

Potential challenges and future perspectives in the use of exosomes

Inherent structural and biocompatible properties of exosomes make them a promising therapeutic and diagnostic tool for many diseases. Nevertheless, many issues and challenges need to be resolved in order to effectively harness the therapeutic potential of exosomes for clinical applications. Although extensive research has provided valuable insights in the field of exosomes, the exact mechanisms of exosome formation, cargo sorting, interaction between exosomal contents and exosome secretion have yet to be elucidated. Likewise, the selection of appropriate exosomes from body fluids and optimal formulation of exosomal carriers are not completely understood. Furthermore, exosomes are only one kind of extracellular vesicle (EV). Therefore, optimization and standardization of isolation/ purification methods for exosomes and other types of EVs remain as notable challenges, as current methods require extensive sample preparation and expensive equipment. The multiple methods currently used for exosome isolation, including differential centrifugation, ultracentrifugation, high-performance liquid chromatography paired with centrifugation, size exclusion chromatography, antibody-based pulldown and polymer-based precipitation, all advantages and disadvantages [127,128].

In addition to appropriate isolation methods, dosage and routes of administration for exosomes remain controversial. Exosomes contain a wide variety of compounds such as proteins, lipids, mRNAs and miRNAs that can serve as diagnostics biomarkers in biological fluids. It is known that exosomal cargos vary under different pathological conditions. An ideal biomarker should be specific, sensitive and predictive for a particular disease. The content and number of exosomal cargos can vary under different pathophysiological conditions. Therefore, more effort should be directed towards identifying specific exosome-associated biomarkers.

Previous studies on exosomal biomarkers have limitations. Most studies simply analyzed exosome number and content at a single time point without continuous measurements. The majority of analyzed only a select number of miRNAs or proteins, without fully covering all the differentially expressed exosomal contents. To address this, more miRNAs and proteins need to be considered as candidate biomarkers for the diagnosis of specific diseases.

Conclusion

This review highlights and summarizes current knowledge regarding the role of exosomes in cardiovascular disease, and with specific emphasis on cardioprotection. The exosomes derived from cardiovascular cells, such as cardiomyocytes, endothelial cells and stem cells, can act as vehicles for the transportation of cytokines, growth factors and miRNAs, which may participate in cardioprotection and/or regeneration post-injury in the tissues of the cardiovascular system. Our review also suggests a novel role for MSC- and CPC-derived exosomes as potential candidates for adjunctive therapy in patients with cardiovascular ischemic diseases.

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